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ISSN: 0974 – 3987
IJBST (2021), 14(4): 44-51

DOI: http://doi.org/10.5281/zenodo.5722748

Bacteriological and Physico-chemical Analyses of domestic well water and rain water in Anambra state, NIGERIA

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ABSTRACT

Water is an important resource needed by man for survival. It serves several purposes ranging from domestic, industrial, agricultural uses amongst others. This research work is aimed at assessing the bacteriological and physic-chemical parameters of domestic rain water and well water in Uli, Anambra state, Nigeria. The water samples were aseptically collected using sterile specimen bottles and their bacterial counts were determined using standard microbiological procedures, Bacteria present in the water samples were isolated and identified based on cultural, morphological characteristics and a battery of biochemical tests. Physico-chemical parameters such as pH, turbidity, salinity, phosphates and nitrates were also determined. It was observed that well water had the highest bacterial counts of 33.30±0.25 cfu/ml, 40.00±1.15 cfuj/ml and 44.20±1.05 cfu/ml in months of May, June and July respectively, when compared to rain water. Bacteria identified from the water samples are E. coli, Klebsiella sp., Streptococcus sp. and Citrobacter sp. It was also noted that the physico-chemical values of the water samples did not vary so highly from the National Food and Drugs Administration Control (NAFDAC) standards. However, it is pertinent that the water samples be treated in order to control their microbial loads and to avoid water related illnesses.

Keywords: coliform, p[H], rain water, total bacterial count, well water.

INTRODUCTION

Water is not only essential to life but it is the predominant inorganic constituent of living matter, forming, in general, nearly three quarters of the weight of the living cell. It makes up to some 5 percent of the body weight of an adult human and can form as much as 98 percent of the mass of certain jellyfish. Organisms which contain relatively small amounts of water are generally in dormant state or show very slow development; seeds and certain invertebrates that live in arid environments are examples. On the other hand, high rainfall over a land mass invariably means a large biomass per unit area [1]. Man uses water not only for drinking purposes but also for bathing, washing, laundering, heating, air conditioning, agriculture, stock raising and gardens.

Water serves as the second natural medium for the growth of microorganisms and stands next to soil. The growth of microorganisms in water mainly depends on the amount of available mineral nutrients and the dissolved oxygen present in it. The number of bacteria and other microbes will always be higher in river passing by thickly populated cities then of the villages because persons living in cities, are continuously disposing sewage water and other waste products in rivers which contain a very high amount of mineral nutrients- a medium for their growth. Moreover, the pH, temperature range and inorganic phosphate content as well as the situation of the lake and river also support the growth and cause a dense population of microorganisms. These organisms (bacteria, blue green algae, etc.) form heavy blooms under these conditions [2].

Because coliform bacteria are most commonly associated with sewage, or surface and groundwater they are used as indicator group to determine the sanitary quality of drinking water [3]. Though most Coliform bacteria do not cause illness, their presence in a water system

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DOI: http://doi.org/10.5281/zenodo.5722748

is a public health concern because it suggests that other disease causing organisms may exist in the water [4]. Coliform bacteria are commonly found in the soil, on vegetation and in water. They are also found in the intestine of warm blooded animals [5]. Coliform from animals wastes can enter directly into water supplies and contaminate the groundwater source thereby rendering it defective. The hand-dug wells are open and exposed to intensive human activity, leading to the likelihood of being contaminated. The contamination of the wells can be caused by both point-source e.g. accidental spills, washings, leakages, dumping of animal wastes etc. and nonpoint-source e.g. runoff from roads, chemicals used in agriculture such as fertilizers, pesticides and herbicides. These sources of pollution may also contribute to the total and fecal coliform contamination thereby raising concerns about its safety; especially concerns about its potential for disease transmission.

Water borne diseases are the most important concern about the quality of water. Safe drinking water is defined by the W.H.O as treated surface or untreated but uncontaminated underground water such as bore-holes, springs and sanitary wells [6]. Water borne disease can cause dysentery, typhoid fever, Salmonellosis and vibrio illness depending on the etiologic agent associated with each infection. Therefore, the quickest ways to prevent outbreak of such diseases and to determine the portability of such water sources is to determine the microbial load or content, if the microbial load is not within acceptable limit, such water sources should be condemned immediately [7].
The study is aimed to analyse the bacteriological and physicochemical quality of well water and rain water in use, from selected local areas around Uli, Anambra state, Nigeria.

MATERIALS AND METHODS

Study Area

Four well water of varied depths and rain water were randomly collected from selected three locations in Uli environs.

Sample collection

Well water samples were collected with sterile 200ml screwed capped glass bottles. The bottle was brought up to the surface and covered with a screw cap with no air bubbles. All the sampled bottles were immediately labelled and transported in ice-pack to the laboratory for bacteriological analysis within 6 hours of collections.

Determination of Bacteria load

A 1 ml aliquot of water sample was serially diluted in 9 ml of nutrient broth, and 0.1 ml aliquot was aseptically aspirated from 10^{-4} tube dilution and spread on sterile nutrient agar plate, incubated for 24 h, thereafter colony counts were taken and bacteria concentration was determined.

Isolation and Characterization of Bacteria Isolates

Gram Staining

A thin smear of the organism was made on a clean microscopic slide. It was air dried and then heat-fixed by passing briefly over flame. Two drops of crystal violet were added to the smear for 1 minute, and then rinsed with clean water. Lugol's Iodine was added for 1 minute and washed. It was then decolorized by flooding with acetone for 30 seconds. The film was rinsed with water and counter stained with Safranin for 10 seconds; it was rinsed with water again and allowed to dry. Microscopic observation was made using oil immersion objective lens [8].

Motility Test

The microorganism was stabbed into a sterilized motility test media (Sigma-Aldrich M1053) contained in a sterile test tube, using a sterile inoculating needle. It was incubated for 18-48 h at 35-37^{0}C and observed for diffused lines of turbidity emerging from the original line of inoculation.

Indole Test

Kovac's reagent (0.5 ml) was added to 5ml of a 48 h peptone water culture of the test organisms. The

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mixture was shaken thoroughly and allowed to stand for 10 minutes [8].

Methyl Red and Voges Proskaeur Reaction

Methyl Red Test

About 3 drops of methyl red indicator were added to 5 ml of 24 h peptone water culture of the test organisms in sterile test tubes. The tubes were incubated for 24 h at 37^{0}C and observed for color change [8].

Voges Proskauer Test

Three drops of alpha –napthol and potassium hydroxide (also called Barrit's reagent B) were added to 5 ml of 24 h peptone water culture of the test organisms in sterile test tubes. The cultures were allowed to settle for about 15 minutes for colour development to occur. A red colour appearance was indicated a positive result, while a yellowish colour indicated a negative result [8].

Citrate Utilization Test

A 0.1 ml aliquot of each test organism was inoculated into Simmon's citrate medium and incubated 48h at 35^{0}C. Colour change from green to blue indicated a positive result, otherwise negative [8].

Catalase Test

A small part of the test colony was collected using a sterile wire loop and immersed into a sterile test tube containing 2-3ml 30% Hydrogen peroxide solution and observed for the appearance of effervescence [8].

Sugar Fermentation Tests

Sugars such as glucose, sucrose, lactose and maltose were added in peptone water in 1% (w/v) and with two drops of Bromothymol blue indicator, and then 1.5 ml aliquot each was distributed in standard assay tubes, each containing an inverted Durham tube. The sugar solutions were sterilized by autoclaving at 115^{0}C for 15 minutes and 200µl of the bacterial samples were inoculated in each tube, and then incubated at 37 °C; color change and gas production was observed after 48 h.

Urease Test

Urease was to determine the ability of the isolates to produce the enzyme urease; which hydrolyses urea forming ammonia and carbon dioxide. 2.4g of urease base agar was dissolved in 100ml of water, heated and 5ml dispensed into tubes and then sterilized at 115°C for 10minutes. Allowed to cool and gel, while still incubating at 37°C for 24hrs. Color change from yellow to red indicate positive result.

Hydrogen sulphide Test

The hydrogen sulphide test was used to determine the ability of the isolates to produce hydrogen sulphide. Test tubes containing 5ml sterile peptone water medium were inoculated with young cultures of the isolates and traps of moist lead acetate paper were place at the opening of the test tubes in such a way that the lower end of the strips were above the liquid medium level but below the end of the cotton plug. The tubes were indicated by the blackening of the tips of the lead acetate papers inside the tubes.

Physico-Chemical Analysis

The physicochemical tests included the determination of p^H, turbidity, Biological Oxygen Demand (BOD), phosphate, electrical conductivity and sulphate, color and nitrite [9].

RESULTS AND DISCUSSION

DISCUSSION

Generally, the well water is believed to be purest because of the purification processes it went through while percolating through the subsurface. However, it can be contaminated [10]. Well water

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and rainwater is found to be contaminated due to improper construction, shallowness and various human activities. In this study, the water assessed from rain water in the month of May had the highest mean bacterial count 20.50±1.02 cfu/ml from sample two, while bacterial counts of 19.50±1.33 cfu/ml and 19.0±0.66 cfu/ml were the highest mean values from samples 6 and 10 in the months of June and July respectively. Well water highest mean bacterial counts from the three months are 33.30±0.25 cfu/ml, 40.00±1.15.88 cfu/ml and 44.20±1.05 cfu/ml from samples 15, 19 and 21 in the months of May, June and July respectively as shown in Table 1.

From the various biochemical identification test carried out, the following organisms were isolated isolated from the samples: *E. coli, Klebsiella* spp, *Citrobacter* spp from well water and *Streptococcus* spp, *Klebsiella* spp from rain water as seen in Table 2. The conducted studies indicated the presence of considerable amounts of pathogenic bacteria in rainwater flowing over different rooftops. In early rainy season they are, however, more compared to later times in the season. The presence of a group of bacteria known as coliforms in water samples serve as indicators of pollution. Chief among them is *Escherichia coli*, which was isolated from the samples used in this study and whose presence indicates the possible presence of other intestinal pathogens. *Streptococcus* spp, *Klebsiella* spp and *Citrobacter* spp, which were isolated and identified from the samples are equally pathogens of importance that have been linked to gastrointestinal disorders. These findings correspond with that of [9].

The overall bacterial count in well water was higher than that gotten from the rain water, which could be attributed to poor environmental hygiene and influx of contaminated flood water which percolate into the underground water, thus getting it contaminated.

According to Krist [10], disposal of solid waste on land contributes largely to ground water pollution. Although treatment of water contaminated by pathogens or sediments is usually expensive, it cannot be compared with the cost of treatment of diseases resulting from the consumption of polluted water. In most cases, children and newborns are the most vulnerable to these water-borne diseases since their immune systems are not as developed as that of adults. Presence of enteric pathogens from the tested rain water samples indicates that the water reservoir or scooping apparatus or animal fecal droppings on the roof could have been possible sources of contamination. It thus becomes necessary that people who use such water source for domestic activity like cooking should be advised to stop or to boil or treat the water appropriately before use in order to avoid water-borne infections like cholera.

Temperature readings of the tested water were about the range of 27^{0}C and 29^{0}C for well water and rain water respectively in the month of May, 27^{0}C for both in the month of June and 27^{0}C and 24.5^{0}C in the month of July for rain water and well water respectively (Table 3). Although the temperatures of the water samples are higher than recommended by the WHO, they can be considered to be normal in the respective environment. pH values were within the range of 7.41-8.25 and there was notable variations in the conductivity, turbidity, alkalinity, nitrite, phosphate and sulphate values. Rain water in the month of May had the highest conductivity of 64 us/cm, well water in the month of May had the highest alkalinity of 132 mg/L, well water in the month of May had the highest nitrite value of 1.2mg/L, rain water in the month of May had the highest phosphate value of 2.3 mg/L while well water in the month of May had highest sulphate value of 10.8 mg/L.

Biological Oxygen Demand (B.O.D) is a measure of the oxygen in the water that is required by the aerobic organisms. Waters with low B.O.D have low nutrient levels; therefore, much of the oxygen remains in the water. Unpolluted, natural waters should have a B.O.D of 5mgL-1 or less [11]. B.O.D values of the water samples ranged from 1.2 mg/L to 2.9 mg/L as shown in (Table 3) and these B.O.D values shows that there was little biological activity in the samples thus safe for consumption since high

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B.O.D values suggests a high number of heterotrophic bacteria in the environment [12].

Table 1: Bacterial Counts of Rain Water and Well Water through Three Months Monitoring

MONTHS		RAIN WATER		WELL WATER
		Mean Bacteria Counts (X 10^6) cfu/ml		Mean Bacteria Counts (x10^6) cfu/ml
MAY	A1	16.6± 2.05	A 13	28.4±0.66
	A2	20.5±1.2	A14	22.5±1.15
	A3	12.5±2.14	A15	33.3±0.25
	A4	21.5±1.1	A16	20.2±1.26
JUNE	B5	12.5±0.33	17	20.0±1.66
	B6	19.5±1.33	18	38.0±0.15
	B7	12.0±1.65	19	40.0±1.15
	B8	13.0±1.48	20	30.0±2.05
JULY	C9	11.5±1.02	21	44.2±1.05
	C10	19.0±0.66	22	36.0±1.50
	C11	11.5±2.14	23	29.6±0.88
	C12	12.3±1.33	24	38.7±2.04

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Table 2: Biochemical Characterization of Isolates from Water Samples

Biochemical Tests	Water Sources					
	Rain		Well			
Colony morphology	Cocci	Rod	Cocci	Rod	Rod	Rod
Gram Stain	+	-	+	-	-	-
Catalase	-	+	-	+	-	-
Indole	-	-	-	-	+	-
Methyl red	-	-	-	-	+	+
Voges prauskeur	+	+	+	+	-	-
Citrate	-	+	-	+	-	-
Urea	-	-	-	-	-	-
Motility	-	-	-	-	+	+
Glucose	+	+	+	+	+	+
Sucrose		+	ND	+	+	+
Mannitol	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Hydrogen Sulphide	-	-	-	-	-	-
Presumptive Organism	*Streptococcus* sp.	*Klebsiella* sp.	*Streptococcus* sp.	*Klebsiella* sp.	*Escherichia coli*	*Citrobacter* Sp.

+ = positive, - = negative, ND = not done.

Table 3: Physicochemical properties of Water Samples

Parameters	RAIN	WELL	RAIN	WELL	RAIN	WELL
Temp	29	27	27	27	27	24.5
Ph	7.8	7.74	7.41	7.63	8.25	7.91
Conductivity(ms/cm)	64	58	52	44	48	37
Turbidity(FTU)	18	22	15	19	14	18
Alkalinity(mg/ml)	103	132	92	94	98	98
nitrite(mg/ml)	0.1	1.2	0.1	0.4	0.2	0.4
Phosphate(mg/ml)	2.3	2.2	1.4	1.8	1.8	1.92
Sulphate(mg/ml)	10.2	10.8	9.7	12.4	6.43	8.9
BOD(mg/ml)	2.5	2.1	2.9	2.8	1.2	2.1
DO(mg/ml)	8.7	7.8	7.4	6.4	8.5	8.9
Real color (units)	40	48	32	66	33	79
Apparent color(units)	63	72	64	74	64	79

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The turbidity of water depends of the quantity of solid matters present in the suspended state. It is a measure of light emitting properties of water and the test is used to indicate the quality of waste discharge with respect to colloidal matter [12]. The turbidity values of the samples were within the environmental protection agency acceptable limits which maybe an indication of no/fewer number of planktons, clay particles and disease causing microorganisms [12].

Phosphate levels in the examined waters were within the permissible limit of 3mg/L. The phosphate values and electrical conductivity values of both water samples were within the permissible limits stated by FAO.

Alkalinity values of the water samples in May were higher than the permissible range approved by NAFDAC which is 100 mg/L.

The pH is one of the most important parameters of water quality. It influences physical and chemical water characteristics (HC 2016). The p^H promotes the solubility of certain substances that are harmful to water quality. The waters sampled are slightly acidic and basic. The p^H were within the permissible range approved by NAFDAC (6.5-8.2) for drinking water. Even though p^H has no direct effect on human health, its indirect action on physiological process cannot be over emphasized [13]. The color of the tested water samples are not in tandem with that approved by NAFDAC which is 15units. The nitrite levels of the tested rain water samples are all within the NAFDAC approved range while that of the tested well water samples are higher than the approved range thus not suitable for human consumption.

CONCLUSION

It has been seen from this research work that the practice of storing rain water poses possible health risks to the consumers in as much as it is a cheaper way of accessing water for domestic use. It has also been shown that well water being an underground water source is exposed to possible contamination as a result of seasonal variations in rainfall.

Concerning water samples from wells, pollution is explained by the fact that animals are drinking at open wells and leave their feces and urine in the vicinity of the wells. However, the samples taken from the wells are excellent physic-chemical quality but of poor bacteriological quality. Thus it should be properly treated before use.

Water purification method that provides safe domestic water should be made available by government in order to avoid outbreak cause by pathogenic organism found in water. The government should make more sacrifices to provide adequate treatment facilities that purify sewage prior to discharge or disposal, so as to save our drinking water from continuous pollution. The community should not compromise on their sanitary practices as a dirty environment could serve as source by which well water gets contaminated.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Microbiology of the University for assistance to use relevant facilities and provision of some materials for this work. Ezemba, C.C. ensured that the bench work of this project was successful.

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